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## Specification

Therapeutic Agent for Glaucoma Comprising Rho Kinase Inhibitor and  $\beta$ -Blocker

#### Technical Field

The present invention relates to a therapeutic agent for glaucoma comprising the combination of a Rho kinase inhibitor and a  $\beta$ -Blocker.

#### Background Art

Glaucoma is an intractable ocular disease with a risk of blindness, involving the increase of intraocular pressure due to various factors and the disorder of internal tissues of eyeballs (retina, an optic nerve and the like). A general method of treating glaucoma is intraocular pressure reduction therapy, which is exemplified by pharmacotherapy, laser therapy, surgical therapy and the like.

For the pharmacotherapy, drugs such as sympathomimetic agents (nonselective stimulants such as epinephrine,  $\alpha_2$  stimulants such as apraclonidine), sympatholytic agents ( $\beta$ -blockers such as timolol, befunolol, carteolol, nifradilol, betaxolol, levobunolol and metipranolol and  $\alpha_1$ -blockers such as bunazosin hydrochloride), parasympathomimetic agents (pilocarpine and the like), carbonic anhydrase inhibitors (acetazolamide and the like) and prostaglandins (isopropyl unoprostone, latanoprost, travoprost, bimatoprost and the

like) have been used.

Recently, a Rho kinase inhibitor was found to serve as a therapeutic agent for glaucoma based on a new mechanism of action (WO 00/09162). Invest. Ophthalmol. & Vis. Sci., 42 (1), 137-144 (2001) discloses that the Rho kinase inhibitor increases the aqueous humor outflow from a trabecular meshwork outflow pathway thereby reducing intraocular pressure, and Invest. Ophthalmol. & Vis. Sci., 42 (1), 137-144 (2001) and Invest. Ophthalmol. & Vis. Sci., 42 (5), 1029-1037 (2001) suggest that the action is due to a change of cytoskeleton in trabecular meshwork cells.

Combined use of drugs having actions of reducing intraocular pressure to treat glaucoma has already been studied and there are some reports on the studies. For example, Japanese Patent No. 2726672 reports combined administration of the sympatholytic agent with prostaglandins. WO 02/38158 discloses a method of treating glaucoma by administering some drugs having actions of reducing intraocular pressure in combination to eyes.

However, any reports do not describe the Rho kinase inhibitor at all, and naturally, there is no description concerning advantageous effects brought about by combining the Rho kinase inhibitor with a  $\beta$ -blocker, either.

As mentioned above, neither study nor report has been made concerning therapeutic effects on glaucoma obtained by combining the Rho kinase inhibitor with the  $\beta$ -blocker, so far.

## Disclosure of the Invention

It is a very interesting subject to find utility as a therapeutic agent for glaucoma due to a combination of a Rho kinase inhibitor and a  $\beta$ -blocker.

Studying precisely effects due to the combination of a Rho kinase inhibitor and a  $\beta$ -blocker, the present inventors found that an action of reducing intraocular pressure is increased and/or persistence of the action is improved by combining these drugs compared with a case where each drug is used alone and consequently completed the present invention. Detailed test methods and their effects are described later under the item of "Pharmacological Tests". A remarkable increase in action of reducing intraocular pressure and/or remarkable improvement of persistence of the action was observed by combining a Rho kinase inhibitor with a  $\beta$ -blocker. The present invention can be suitably used for treating and preventing ophthalmopathy accompanied by a rise of intraocular pressure.

The increase in the action of reducing intraocular pressure and/or persistence of the action is improved by administering the combination of the Rho kinase inhibitor and the  $\beta$ -blocker to eyes. Accordingly, the present invention is useful as a therapeutic agent for glaucoma.

The present invention relates to a therapeutic agent for glaucoma comprising the combination of a Rho kinase inhibitor and a  $\beta$ -blocker. These drugs complement and/or enhance their actions each other.

For the method of administration, each of the Rho kinase inhibitor and the  $\beta$ -blocker can be in a separate preparation and these drugs can be administered in combination. Alternatively, these drugs can be formulated in a single preparation to be administered. In other words, these drugs can be administered in mixture.

The Rho kinase inhibitors and the  $\beta$ -blockers of the present invention include salts thereof. When these compounds have a basic group such as an amino group, they can be salts with an inorganic acid such as hydrochloric acid or nitric acid or with an organic acid with oxalic acid, succinic acid, acetic acid or maleic acid. When they have an acidic group such as a carboxyl group, they can be salts with an alkali metal such as sodium or potassium or with an alkaline earth metal such as calcium.

The Rho kinase inhibitors and the  $\beta$ -blockers of the present invention include derivatives thereof such as esters. Specific examples of esters are alkyl esters such as methyl esters, ethyl esters and isopropyl esters.

Further, the Rho kinase inhibitors and the  $\beta$ -blockers of the present invention can be in the form of hydrates or solvates.

The present invention is characterized by treating glaucoma with the combination of a Rho kinase inhibitor and a  $\beta$ -blocker.

The Rho kinase inhibitor in the present invention means a compound which inhibits serine/threonine kinase activated with activation of Rho. Examples of such compounds are the compounds which inhibit ROK $\alpha$  (ROCK-II), p160ROCK (ROK $\beta$ , ROCK-I) and

other compounds which inhibit proteins having a serine/threonine kinase activity. Specific Rho kinase inhibitors are exemplified by Rho kinase inhibitors such as (R)-trans-N-(pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide and

(R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)-benzamide disclosed in WO 98/06433 and WO 00/09162, Rho kinase inhibitors such as 1-(5-isoquinolinesulfonyl)homopiperazine and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine disclosed in WO 97/23222 and Nature, 389, 990-994 (1997), Rho kinase inhibitors such as (1-benzylpyrrolidin-3-yl)-(1H-indazol-5-yl)amine disclosed in WO 01/56988, Rho kinase inhibitors such as (1-benzylpiperidin-4-yl)-(1H-indazol-5-yl)amine disclosed in WO 02/100833, Rho kinase inhibitors such as N-[2-(4-fluorophenyl)-6,7-dimethoxy-4-quinazolinyl]-N-(1H-indazol-5-yl)amine disclosed in WO 02/076976, Rho kinase inhibitors such as N-4-(1H-indazol-5-yl)-6,7-dimethoxy-N-2-pyridin-4-yl-quinazolin-2,4-diamine disclosed in WO 02/076977 and Rho kinase inhibitors such as 4-methyl-5-(2-methyl-[1,4]diazepan-1-sulfonyl)isoquinoline disclosed in WO 99/64011.

On the other hand, any  $\beta$ -blockers having the action of reducing intraocular pressure and utility in treating glaucoma can be used.  $\beta$ -Blockers having the action of reducing intraocular pressure are specifically exemplified by timolol, befunolol, carteolol, nipradilol, betaxolol, levobunolol and metipranolol, which have already been on

the market as a therapeutic agent of glaucoma. These are preferably used.

Glaucoma in the present invention includes primary open angle glaucoma, normal intraocular tension glaucoma, hypersecretion glaucoma, ocular hypertension, acute angle-closure glaucoma, chronic closed angle glaucoma, combined-mechanism glaucoma, corticosteroid glaucoma, amyloid glaucoma, neovascular glaucoma, malignant glaucoma, capsular glaucoma, plateau iris syndrome and the like.

To carry out the present invention, preparations can be two preparations prepared by formulating a Rho kinase inhibitor and a  $\beta$ -blocker separately or one preparation prepared by mixing these ingredients. Particular techniques are unnecessary for the formulation, and the preparations can be prepared using widely-used techniques. A preferred method of administration is eye topical administration, and a preferred dosage form is an ophthalmic solution or an eye ointment.

When a Rho kinase inhibitor and a  $\beta$ -blocker are formulated in preparations separately, each preparation can be prepared according to known methods. For example, the Rho kinase inhibitor can be formulated in preparations by referring to Formulation Examples described in the above-mentioned International Publications (WO 00/09162 and WO 97/23222). As the preparations of the  $\beta$ -blocker, preparations of timolol, befunolol, carteolol, nipradilol, betaxolol, levobunolol, metipranolol and the like can be used. These preparations have already been on the market as the therapeutic agent of glaucoma.

The formulation containing a Rho kinase inhibitor and a

$\beta$ -blocker in mixture can be also prepared according to known methods. The ophthalmic solutions can be prepared, using isotonic agents such as sodium chloride and concentrated glycerin; buffers such as sodium phosphate buffer and sodium acetate buffer; surfactants such as polyoxyethylene sorbitan monooleate, stearate polyoxyl 40, and polyoxyethylene hardened castor oil; stabilizers such as sodium citrate and sodium edetate; and preservatives such as benzalkonium chloride and paraben, as needed.

The pH should be within an ophthalmologically acceptable range and is preferably within a range from pH 4 to pH 8. For reference, a formulation example thereof is described below in the section of Example. However, the formulation example never limits the scope of the invention.

The present invention also relates to a method of treating glaucoma comprising administering effective amounts of the Rho kinase inhibitor in combination with the  $\beta$ -blocker to a patient. By administering the effective amounts of the Rho kinase inhibitor in combination with the  $\beta$ -blocker to the patient, they complement and/or enhance their actions each other. Rho kinase inhibitors which are suitable for the method of treatment are (R)-trans-N-(pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide, (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide, 1-(5-isoquinolinesulfonyl)homopiperazine and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine.  $\beta$ -Blockers which are suitable for the method of treatment are timolol, befunolol, carteolol,

nipradilol, betaxolol, levobunolol and metipranolol.

The doses of a Rho kinase inhibitor and a  $\beta$ -blocker can be determined depending on the symptom and age of patients, the dosage form, the administration route and the like. The case of instillation is briefly described below. The dose of the Rho kinase inhibitor varies depending on the drug type. The Rho kinase inhibitor can be administered generally within a range from 0.025 to 10,000  $\mu\text{g}$  daily from once to several times a day. The dose can be appropriately raised or lowered depending on the age and symptom of patients and the like.

The dose of a  $\beta$ -blocker varies depending on drug type. The usual daily dose is within a range from 5 to 5,000  $\mu\text{g}$ , which can be administered from once to several times a day. More specifically, timolol is generally administered at a daily dose of 5 to 1,500  $\mu\text{g}$ , befunolol is administered at a daily dose of 10 to 2,000  $\mu\text{g}$ , carteolol is administered at a daily dose of 10 to 5,000  $\mu\text{g}$ , nipradilol is administered at a daily dose of 10 to 1,250  $\mu\text{g}$ , betaxolol is administered at a daily dose of 50 to 1,000  $\mu\text{g}$ , levobunolol is administered at a daily dose of 5 to 5,000  $\mu\text{g}$ , and metipranolol is administered at a daily dose of 5 to 5,000  $\mu\text{g}$ . Depending on the age, symptoms and the like of patients, the doses can be varied. Based on similar standards, the doses of the other  $\beta$ -blockers can be determined.

These doses are also applicable to the administration of the combination of a Rho kinase inhibitor and a  $\beta$ -blocker. In case that a Rho kinase inhibitor and a  $\beta$ -blocker are to be administrated in one formulation, the formulation should be prepared by selecting the

mixing ratio of two drugs appropriately so that their daily doses might not exceed each dose of the separate drugs. The mixed formulation can be administered from once to several times daily.

#### Brief Description of Drawings

Fig. 1 is a graph showing changes of intraocular pressure with time in respective administration groups.

#### Best Mode for Carrying out the Invention

A formulation example and pharmacological tests are shown in the following Examples. The Examples are for better understanding of the invention but never limits the scope of the invention.

#### Examples

##### Formulation Example

A general formulation example of an ophthalmic solution comprising a Rho kinase inhibitor ((R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide dihydrochloride) and a β-blocker (timolol) in the present invention is shown below.

##### Ophthalmic solution (in 100mL)

(R)-(+)-N-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide dihydrochloride	0.1 g
Timolol maleate	0.34 g
Boric acid	0.2 g
Concentrated glycerin	0.25 g

Benzalkonium chloride	0.005 g
Diluted hydrochloric acid	quantum sufficient
Sodium hydroxide	quantum sufficient
Purified water	quantum sufficient

Ophthalmic solutions having desired combinations and desired concentrations can be prepared by changing the kinds and amounts of a Rho kinase inhibitor and a  $\beta$ -blocker and by appropriately changing the amounts of the additives.

#### Pharmacological tests

So as to study the utility of the combination of a Rho kinase inhibitor and a  $\beta$ -blocker, they were administered to experimental animals, examining the effect on reducing intraocular pressure. (R)-(+)-N-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide dihydrochloride [Compound A] was used as the Rho kinase inhibitor. Timolol was used as the  $\beta$ -blocker.

#### Preparation of test compound solutions

##### 1. Preparation of Rho kinase inhibitor solutions

The Rho kinase inhibitor was dissolved in physiological saline, and then sodium hydroxide was added to the solution to neutralize it (pH 6.0 to 7.0) to thereby prepare Rho kinase inhibitor solutions having desired concentrations.

##### 2. Preparation of $\beta$ -blocker solutions

A commercially available  $\beta$ -blocker ophthalmic solution was

used as it was, or was diluted with physiological saline to prepare  $\beta$ -blocker solutions having desired concentrations.

#### Method of test

Administering the combination of the Rho kinase inhibitor and the  $\beta$ -blocker, the effect on reducing intraocular pressure was studied. As a reference, administering the Rho kinase inhibitor alone or the  $\beta$ -blocker alone, the effect on reducing intraocular pressure was also studied. As a control, only a vehicle (physiological saline) was administered.

#### Drugs and animals to be used for tests

Rho kinase inhibitor solution: 0.1% compound A solution (instillation dosage: 50  $\mu$  l)

$\beta$ -Blocker solution: Timolol ophthalmic solution (trade name: Timoptol 0.25%, instillation dosage: 50  $\mu$  l)

Experimental animal: Japanese white rabbit (strain: JW, sex: male, four rabbits per group)

#### Method of administration and method of measurement

1. Administration of the combination of a Rho kinase inhibitor and a  $\beta$ -blocker

1) One drop of a 4% oxybuprocaine hydrochloride ophthalmic solution (trade name: Benoxil solution 0.4%) was instilled into both eyes of each experimental animal to anesthetize it topically.

2) Intraocular pressure was measured immediately before administering the test compound solution, and the intraocular pressure was referred to as initial intraocular pressure.

3) The Rho kinase inhibitor solution was instilled into one eye of each experimental animal (the other eye was not treated). Since it is impossible to instill the  $\beta$ -blocker solution at the same time, after a short period (about five minutes), the  $\beta$ -blocker solution was instilled into the same eye.

4) One, two and four hours after instilling the Rho kinase inhibitor solution, one drop of the 0.4% oxybuprocaine hydrochloride ophthalmic solution was instilled into both eyes to anesthetize them topically. Then intraocular pressure was measured three times to obtain the average of three measurements.

## 2. Single administration of a Rho kinase inhibitor

Each test was carried out in the same manner as in the above-mentioned combination administration test except that the  $\beta$ -blocker solution was replaced with physiological saline.

## 3. Single administration of a $\beta$ -blocker

Each test was carried out in the same manner as in the above-mentioned combination administration test except that the Rho kinase inhibitor solution was replaced with physiological saline.

## 4. Control

Each test was carried out in the same manner as in the above-mentioned combination administration test except that the Rho kinase inhibitor solution and the  $\beta$ -blocker solution were replaced with physiological saline.

## Results and consideration

Results of the tests are shown in Fig. 1. Intraocular pressure is

expressed in each change from initial intraocular pressure.

As apparent from Fig. 1, the Rho kinase inhibitor and  $\beta$ -blocker combination group exhibited an excellent action of reducing intraocular pressure compared with single administration groups of each drug, namely the single administration group of the Rho kinase inhibitor and the single administration group of the  $\beta$ -blocker, and exhibited improvement of persistence of the action.

When combinations of the other Rho kinase inhibitors and the other  $\beta$ -blockers disclosed in the specification were tested, tendencies similar to the above-mentioned test results were also observed.

The above-mentioned results show that a stronger reducing effect on intraocular pressure and/or improvement of persistence is obtained by combining the Rho kinase inhibitor with the  $\beta$ -blocker.

#### Industrial Applicability

The present invention provides a therapeutic agent for glaucoma comprising the combination of a Rho kinase inhibitor and a  $\beta$ -blocker.